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(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) Publication number : **0 670 494 A2**

(12) EUROPEAN PATENT APPLICATION

(21) Application number : **95400423.0**

(22) Date of filing : **28.02.95**

(51) Int. Cl.[6] : **G01N 33/53**, G01N 33/569, C07K 14/20, C07K 19/00

(30) Priority : **28.02.94 JP 54672/94**

(43) Date of publication of application : **06.09.95 Bulletin 95/36**

(84) Designated Contracting States : **AT BE CH DE DK ES FR GB GR IE IT LI LU MC NL PT SE**

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(54) Anti-treponema pallidum antibody immunoassay.

(57) A new method for assaying anti-Treponema pallidum antibody with improved sensitivity is provided. Anti-Tp antibody in serum is assayed by enzyme immunoanalysis utilizing the antigen-antibody reaction between G15 and/or G17 antigen, a protein available on fusion of GST to the N-terminal of a Tp membrane antigen (15 and/or 17 kDa), and anti-Tp antibody in a sample.

EP 0 670 494 A2

FIELD OF THE INVENTION

This invention relates to a method for detection and assay of anti-Treponema pallidum antibody.

BACKGROUND OF THE INVENTION

Syphilis is a disease caused by Treponema pallidum (hereinafter sometimes referred to briefly as Tp). The diagnosis of syphilis is generally made by an immunoassay of anti-Tp antibody in the blood. The surface of Tp cell has a large number of membrane antigens and the above mentioned immunoassay utilizes the antigen-antibody reaction between the membrane antigen and the anti-Tp antibody in a blood specimen. The Tp membrane antigen for use in the immunoassay today is prepared by innoculating Tp into rabbit testis and disrupting the harvested viable cells. However, since Tp is cultured using rabbit testis, the sensitivity and reproducibility of this assay are low owing to contamination with impurities and, it is difficult to provide a large quantity of Tp.

To overcome these disadvantages, it has been proposed to clone a gene coding for the Tp membrane antigen, mass-produce said membrane antigen by biotechnology and use it in the above mentioned immunoassay. For example, Japanese Kohyo publication Hei-2-500403 describes a technology which comprises preparing a Tp antigen having a molecular weight (MW) of 47kDa by biotechnology and assaying the anti-Tp antibody immunologically using this antigen.

The technology disclosed in Japanese Kohyo publication Hei-2-500403 enables the immunoassay of anti-Tp antibody, but it would benefit the diagnosis of syphilis should the anti-Tp antibody be assayed with improved sensitivity.

Therefore, this invention has for its object to provide a method for assay of anti-Tp antibody which enables the assay of anti-Tp antibody with improved sensitivity as compared with the conventional method.

SUMMARY OF THE INVENTION

The inventors of this invention did much research and found that when G15 antigen and/or G17 antigen which is available on fusion of glutathione-S-transferase (GST) to the N-terminal of a MW 15kDa or 17kDa antigen, among Tp membrane antigens, is used as the assay antigen, anti-Tp antibody can be assayed with a significantly higher sensitivity than the sensitivity of the conventional method.

Thus, this invention provides a method for assay of anti-Tp antibody which comprises quantitating anti-Tp antibody in a sample utilizing the antigen-antibody reaction between either G15 antigen, which is a protein available on fusion of GST to the N-terminal of a Tp membrane antigen having a molecular weight of 15kDa, and/or G17 antigen, which is a protein available on fusion of GST to the N-terminal of a Tp membrane antigen having a molecular weight of 17kDa, and said anti-Tp antibody in a sample.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 is a diagrammatic representation of the structures of G15 and G17 antigens for use in the method of this invention.

Fig. 2 is a graph showing the results of sandwich ELISA according to the method of this invention using G15 and G17 as antigens in comparison with the results of the comparable assay using $47C_4$.

Fig. 3 is a graph showing the results of sandwich ELISA according to the method of this invention using G17 antigen in comparison with the comparable assay using GST-free Mature 17 antigen which was obtained in E. coli expression system as a form close to native antigen.

Fig. 4 is a graph showing the results of sandwich ELISA using a recombinant $47C_2$ antigen in comparison with the results of the same assay using native 47 antigen which was purified from Tp innoculated testis.

Fig. 5 is a graph showing the results of sandwich ELISA using various dilutions of test serum in accordance with the method of this invention.

Fig. 6 is a graph showing the results of sandwich ELISA for various dilutions of test serum using 3 kinds of recombinant 47kDa antigens.

Fig. 7 is a graph showing the results of sandwich ELISA according to the method of this invention in 35 different Tp-positive sera.

DETAILED DESCRIPTION OF THE INVENTION

This invention is now described in detail.

It is known that antigens having various molecular weights are present on the surface of Tp cell, and as

representative species, antigens having molecular weights of 47kDa, 42kDa, 17kDa and 15kDa, respectively, are known. These antigens are distinguishable from one another electrophoretically (Journal of Immunology, Vol. 129, pp.1287-1291, 1982; Journal of Clinical Microbiology, Vol. 21, pp.82-87, 1985; Journal of Clinical Microbiology, Vol. 30, pp.115-122, 1992). In addition to the gene coding for the membrane antigen having a molecular weight of 47kDa, genes coding for membrane antigens having molecular weights of 17kDa and 15kDa have already been cloned and produced by biotechnology. Furthermore, their amino acid sequences have also been established (INFECTION AND IMMUNITY, Vol. 57, No. 12, pp.3708-3714, 1989; Molecular Microbiology (1990) 4(8), 1371-1379; INFECTION AND IMMUNITY, Vol. 61, No. 4 pp.1202-1210, 1993).

The antigen for use in accordance with this invention is a fused protein (G15 antigen) available on fusion of GST to the N-terminal of the above mentioned Tp membrane antigen having a molecular weight of 15kDa, and/or a fused protein (G17 antigen) available on fusion of GST to the N-terminal of the above mentioned Tp membrane antigen having a molecular weight of 17kDa. GST fused proteins as such and the biotechnology of their production are already known (INFECTION AND IMMUNITY, Apr. 1993, pp.1202-1210). However, the inventors of this invention should be credited with the surprising discovery that the sensibility of the immunoassay of anti-Tp antibody is significantly enhanced when either G15 or G17, or both, are used in lieu of the membrane antigens not linked to GST.

The structures of G15 and G17 antigens for use in the practice of this invention are shown in Fig. 1. While these G15 and G17 antigens can be prepared by the methods described in the above mentioned literature, the preferred procedures are described in detail in the examples which appear hereinafter.

The method of this invention is a method for immunoassay of anti-Tp antibody utilizing the antigen-antibody reaction between either said G15 antigen or said G17 antigen, or both, and anti-Tp antibody in a sample. A variety of immunoassay procedures are known to those skilled in the art and any of such known procedures can be utilized in the practice of this invention. Thus, classified by reaction pattern, the sandwich method and the agglutination test, among others, can be utilized. Classified by label, the enzyme immunoanalysis, immunofluorescence analysis and radio immunoanalysis, among others, can be mentioned. Of these methods, the sandwich method (sandwich ELISA) which does not require large-scale equipment and is simple procedure-wise, sufficiently sensitive, and suited for the analysis of multiple samples is the most preferred. For the determination of human anti-Tp antibody, for instance, G15 antigen or G17 antigen, or both, are immobilized in the wells of a 96-well microtiter plate. After blocking the non-specific adhesion sites, the wells are washed and the test sample is added for reaction. The wells are washed and an enzyme (e.g. peroxidase)-labeled anti-human immunoglobulin antibody is added for reaction. The plate is washed and the substrate for the conjugated enzyme is added for enzymatic reaction and color development. After the reaction is stopped, the intensity of the developed color is assessed spectrometrically to quantitate the anti-Tp antibody in the sample. The sandwich ELISA itself is well known in this field and a more detailed description of the procedure can be found in the examples which appear hereinafter. It should be understood, however, that the assay method of this invention is not limited to the sandwich ELISA but includes other assay methods, for instance, an agglutination assay which comprises immobilizing G15 antigen and/or G17 antigen on a particulate carrier such as latex particles, gelatin particles, ferrite particles and the like and adding the test sample to a suspension of the particles to see whether agglutination of the particles would take place.

When said sandwich ELISA is employed, the assay sensitivity can be further improved by calculating the difference of the absorbance between the wavelength used for measuring the developed color and the absorbance at a wavelength at which no color is detected. For example, as shown in the examples given hereinafter, when a peroxidase is used as the conjugated enzyme and ABTS (2,2'-Azino-bis(3-ethylbenzothiozoline-6-sulfonic acid) is used as the substrate, the assay sensitivity can be further improved by measuring the differencial absorbance between 405 nm and 492 nm, i.e. the subtraction of the absorbance at 405 nm from the absorbance at 492 nm.

It should also be understood that the method of this invention can be carried out using either G15 alone or G17 alone or both concurrently as the antigen. The latter practice is preferred because the assay sensitivity is further improved.

By the method of this invention, anti-Tp antibody can be assayed and the diagnosis of syphilis be made accordingly. The test sample that can be used includes various body fluids, such as serum, from man and other animals requiring a diagnosis of syphilis and appropriate dilutions thereof.

In accordance with this invention, anti-Tp antibody can be assayed with improved sensitivity as compared with any of the hitherto-proposed techniques.

EXAMPLES

The following examples are intended to describe this invention in further detail. It should, of course, be

understood that this invention is by no means limited to these specific examples.

Reference Example 1 Preparation of G15 and G17 antigens

Treponema pallidum was purified from a rabbit testis in which Tp was innoculated and cultured and the genomic DNA was extracted. Using this as a template as well as the primers prepared based on the known DNA sequence using a DNA synthesizer, DNA fragments corresponding to 15k and 17k antigens were prepared by the PCR method. Each of these DNA fragments did not include the 5'-end DNA sequence corresponding to the N-terminal peptide cleaved off in the native antigen. Into the vector pWG6A constructed for expression of GST-fused protein, these DNA fragments were respectively inserted and the resulting vectors were named pWG6A-15k and pWG6A-17k, respectively. Each of them was introduced into Escherichia coli to induce the expression of G15 or G17 antigen. After expression of the antigen, the E. coli was sonicated and centrifuged and the supernatant was serially purified by DEAE-Sepharose (Whatman DE52) ion exchange chromatography and phenyl-Sepharose CL4B (Pharmacia) hydrophobic chromatography. The final yields of G15 and G17 antigens from 1-liter cultures were approximately 60 mg and their purities were not less than 95 %. The amino acid sequences of G15 antigen and G17 antigen so obtained are respectively SEQ ID N° 1 and SEQ IS N° 2. Each of the resulting G15 and G17 antigens was subjected to SDS-polyacrylamide electrophoresis and further to Western blotting using rabbit anti-Tp serum as a primary antibody, whereupon an intense color was developed. On the other hand, Treponema pallidum purified from rabbit testis was subjected to SDS-polyacrylamide electrophoresis and to Western blotting using the anti-G15 and anti-G17 monoclonal antibodies constructed by sensitizing mice with G15 and G17 antigens, respectively, as primary antibodies. As a consequence, an intense color developed with the respective native 15k antigen and 17k antigen, indicating that immunologically the recombinant G15 and G17 antigens were equivalent to the native antigens 15k and 17k.

Example 1

In the wells of a plastic plate (Falcon 39 Micro Test 111 Flexible assay plate), the G15 antigen or G17 antigen prepared in Reference Example 1 was immobilized at various sensitizing concentrations. As control, $47C_4$ antigen (Amino acid number 1, Met to 434, Gln), a Tp membrane antigen having a molecular weight of 47kDa, was prepared in accordance with the method of Norgard et al. (INFECTION AND IMMUNITY Apr. 1992, pp.1568-1576), purified by electrophoresis, and immobilized in the other wells at various sensitizing concentrations. After blocking of the non-specific adhesion sites with 5% Skim Milk, a commercial blocking agent, the wells were washed and 50 µl of the test sample was added. The test sample was prepared by diluting a syphilis patient's serum 500-fold with 10 mM PBS containing 1% skim milk and 0.05% Tween 20 (tradename). After addition of the test sample, the plate was incubated at 37°C for 1.5 hours. The wells were then washed and 50 µl of peroxidase-conjugated anti-human IgG solution was added. The plate was incubated at 37°C for 1.5 hours and, then, washed. Then, 50 µl of a solution of the color developer ABTS was added and the plate was incubated for 30 minutes. The reaction was stopped with 100 µl of oxalic acid and the absorbances at 405 nm and 492 nm were measured with a spectrophotometer (Titerteck Multiskan Plus). The results are shown in Fig. 2.

It can be seen from Fig. 2 that with the absorbance found with the $47C_4$ antigen being taken as unity, the assay sensitivity at the sensitizing antigen concentration of 10 µ g/ml is about 6-fold as high with the G15 antigen and about 4-fold as high with G17 antigen. Thus, compared with the use of $47C_4$ antigen, significantly higher assay sensitivities can be obtained with G15 and G17 antigens.

Example 2

Using the G17 antigen prepared in Reference Example 1 and, as control, the Mature 17 antigen having a molecular weight of 17kDa (prepared by the method of Norgard et al. (INFECTION AND IMMUNITY, Apr. 1993, pp.1202-1210) and purified by anion exchange chromatography (DE52) and affinity chromatography using anti-17k monoclonal antibody), the assay of anti-Tp antibody was carried out in the same manner as Example 1. the results are shown in Fig. 3. It can be seen from Fig. 3 that compared with Mature 17 antigen (Amino acid number 22, Cys to 156, Lys), G17 antigen provided for the half value of maximum absorbance at about one-tenth of the concentration. It was, thus, confirmed that a significantly higher assay sensitivity can be obtained with G17 antigen carrying GST as compared with the corresponding Mature antigen.

Reference Example 2

Assays were carried out in the same manner as in Example 1 using the conventional 47kDa antigen partially purified (purity ≧ 80%) from Tp (native 47 antigen) by the conventional method and the corresponding recombinant antigen ($47C_2$, Amino acid number 21, Gly to 434, Gln, prepared by the method disclosed in Japanese Kohyo pulbication Hei-2-500403).

The results are shown in Fig. 4.

It is apparent from Fig. 4 that there is no remarkable difference in assay sensitivity between the case of using native 47 and the case of using $47C_2$ prepared by recombinant DNA technology (although the native 47 was somewhat more sensitive; the vertical scale of Fig. 4 is much different from the scale of Figs. 2 and 3 with the result that even a minor difference is apparently exaggerated). Comparison of Fig. 4 with Fig. 2 suggests that the sensitivity of the method of this invention employing G15 or G17 antigen is higher than the sensitivity of the conventional method using the native antigen derived from Tp.

Example 3

Using plastic plates whose wells had been sensitized with G15 antigen, G17 antigen, and $47C_2$ antigen described in Reference Example 2 at a uniform concentration of 10 μg/ml and various dilutions of test serum, the immunoassay was carried out in the same manner as in Example 1. The results are shown in Fig. 5.

It is apparent from Fig. 5 that the amount of serum required for the assay sensitivity equivalent to that of $47C_2$ antigen was about 1/10 for G15 antigen and about 1/6 for G17 antigen. It was, thus, made clear again that the sensitivity of the method of this invention employing G15 antigen or G17 antigen is higher than the sensitivity of the method using $47C_2$ as well as native 47K (see Fig. 4).

Reference Example 3

Using 3 kinds of 47kDa recombinant antigens, namely 47 antigen (Amino acid number 68, Met to 434, Gln, prepared by the method of Norgard et al.(INFECTION AND IMMUNITY, Apr. 1992, pp.1568-1576) and purified by hydroxyapatite gel filtration), $47C_2$ antigen and $47C_4$ antigen, the wells of plastic plates were sensitized with 5 μg/ml each. Then, using various dilutions of test serum, the assay was performed in the same manner as in Example 1. The results are shown in Fig. 6.

It is apparent from Fig. 6 that there is no remarkable difference in assay sensitivity among these antigens.

In addition, even though a GST fused antigen($G47C_2$) was used for ELISA, there was no significant difference between $G47C_2$ and $47C_2$, i.e. the mean value of the ratio between $G47C_2$ and $47C_2$ was 0.46 ± 0.45 (Mean± SD)(n=35 serum samples). Comparison of Fig. 6 with Figs. 2 and 5 suggests that the method of this invention provides for a higher assay sensitivity than any known 47kDa antigen.

Example 4

Using a plastic plate sensitized with 5 μg/ml of G15 antigen, 1 μg/ml of G17 antigen or 5 μg/ml of $47C_2$ antigen, 500-fold dilutions of 35 Tp-positive sera (verified to be seropositive by the conventional method) and Tp-negative sera were tested in the same manner as in Example 1. The results are shown in Fig. 7 and Table 1.

Table 1

Sample Number	S/N Ratio		
	G15	G17	47C2
1	37.90	142.60	4.53
2	39.60	149.00	8.37
3	47.10	148.68	1.92
4	48.50	127.33	6.58
5	28.60	94.10	2.82
6	39.90	126.66	1.80
7	15.90	86.43	3.88
8	6.71	9.26	1.73
9	59.28	156.30	3.21
10	37.17	114.66	1.19
11	20.73	81.43	3.76
12	32.99	76.26	1.35
13	63.86	183.66	11.90
14	43.14	145.00	5.19
15	37.08	94.33	2.97
16	39.96	118.66	2.15
17	16.58	13.36	3.50
18	63.00	144.66	8.39
19	57.57	169.00	7.60
20	62.00	153.33	11.66
21	36.27	121.66	4.68
22	53.14	152.66	6.47
23	44.28	140.66	5.40
24	37.86	151.33	7.26
25	63.43	164.66	6.66
26	62.86	167.66	4.74
27	38.58	112.00	8.01
28	43.28	165.86	7.17
29	53.57	113.00	8.27
30	37.21	106.00	3.72
31	68.28	95.36	6.83
32	53.40	139.66	6.33
33	41.28	137.66	2.72
34	64.71	146.53	16.23
35	39.54	52.20	12.60

As can be seen from Fig. 6, the order of response of seropositive cases was G15, G17>>47C_2 excepting 3 out of 35 cases. The S/N ratio, i.e. said response (S) divided by response (N) of seronegative cases, is shown as the indicator of relative assay sensitivity in Table 1. The order of S/N ratio was, also, G17, G15>>47C_2 and it was confirmed that the use of G15 or G17 antigen provides for significantly higher sensitivity, i.e. about 10-fold (3.1 to 31.2 times) as high in terms of mean G15/47C_2 ratio and about 30-fold (3.8 to 96.4 times) as high in terms of G17/47C_2 ratio.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

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(F) POSTAL CODE (ZIP): 163-07

(ii) TITLE OF INVENTION: Anti-treponema pallidum antibody immunoassay

(iii) NUMBER OF SEQUENCES: 2

(iv) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30 (EPO)

(vi) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: JP 6-54672

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 352 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

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Met Ser Pro Ile Leu Gly Tyr Trp Lys Ile Lys Gly Leu Val Gln Pro
1               5                  10                  15
Thr Arg Leu Leu Leu Glu Tyr Leu Glu Glu Lys Tyr Glu Glu His Leu
            20                  25                  30
Tyr Glu Arg Asp Glu Gly Asp Lys Trp Arg Asn Lys Lys Phe Glu Leu
        35                  40                  45
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Gly Leu Glu Phe Pro Asn Leu Pro Tyr Tyr Ile Asp Gly Asp Val Lys
    50                  55                  60
Leu Thr Gln Ser Met Ala Ile Ile Arg Tyr Ile Ala Asp Lys His Asn
65                  70                  75                  80
Met Leu Gly Gly Cys Pro Lys Glu Arg Ala Glu Ile Ser Met Leu Glu
                85                  90                  95
Gly Ala Val Leu Asp Ile Arg Tyr Gly Val Ser Arg Ile Ala Tyr Ser
            100                 105                 110
Lys Asp Phe Glu Thr Leu Lys Val Asp Phe Leu Ser Lys Leu Pro Glu
        115                 120                 125
Met Leu Lys Met Phe Glu Asp Arg Leu Cys His Lys Thr Tyr Leu Asn
    130                 135                 140
Gly Asp His Val Thr His Pro Asp Phe Met Leu Tyr Asp Ala Leu Asp
145                 150                 155                 160
Val Val Leu Tyr Met Asp Pro Met Cys Leu Asp Ala Phe Pro Lys Leu
                165                 170                 175
Val Cys Phe Lys Lys Arg Ile Glu Ala Ile Pro Gln Ile Asp Lys Tyr
            180                 185                 190
Leu Lys Ser Ser Lys Tyr Ile Ala Trp Pro Leu Gln Gly Trp Gln Ala
        195                 200                 205
Thr Phe Gly Gly Gly Asp His Pro Pro Lys Ser Asp Leu Val Pro Arg
    210                 215                 220
Gly Ser Glu Leu Cys Ser Phe Ser Ser Ile Pro Asn Gly Thr Tyr Arg
225                 230                 235                 240
Ala Thr Tyr Gln Asp Phe Asp Glu Asn Gly Trp Lys Asp Phe Leu Glu
                245                 250                 255
Val Thr Phe Asp Gly Gly Lys Met Val Gln Val Val Tyr Asp Tyr Gln
            260                 265                 270
His Lys Glu Gly Arg Phe Lys Ser Gln Asp Ala Asp Tyr His Arg Val
        275                 280                 285
Met Tyr Ala Ser Ser Gly Ile Gly Pro Glu Lys Ala Phe Arg Glu Leu
    290                 295                 300
Ala Asp Ala Leu Leu Glu Lys Gly Asn Pro Glu Met Val Asp Val Val
305                 310                 315                 320
Thr Gly Ala Thr Val Ser Ser Gln Ser Phe Arg Arg Leu Gly Arg Ala
                325                 330                 335
Leu Leu Gln Ser Ala Arg Arg Gly Glu Lys Glu Ala Ile Ile Ser Arg
            340                 345                 350
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(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 362 amino acids
(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

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1               5                  10                  15
Thr Arg Leu Leu Leu Glu Tyr Leu Glu Glu Lys Tyr Glu Glu His Leu
            20                  25                  30
Tyr Glu Arg Asp Glu Gly Asp Lys Trp Arg Asn Lys Lys Phe Glu Leu
        35                  40                  45
Gly Leu Glu Phe Pro Asn Leu Pro Tyr Tyr Ile Asp Gly Asp Val Lys
    50                  55                  60
Leu Thr Gln Ser Met Ala Ile Ile Arg Tyr Ile Ala Asp Lys His Asn
65                  70                  75                  80
Met Leu Gly Gly Cys Pro Lys Glu Arg Ala Glu Ile Ser Met Leu Glu
                85                  90                  95
Gly Ala Val Leu Asp Ile Arg Tyr Gly Val Ser Arg Ile Ala Tyr Ser
            100                 105                 110
Lys Asp Phe Glu Thr Leu Lys Val Asp Phe Leu Ser Lys Leu Pro Glu
        115                 120                 125
Met Leu Lys Met Phe Glu Asp Arg Leu Cys His Lys Thr Tyr Leu Asn
    130                 135                 140
Gly Asp His Val Thr His Pro Asp Phe Met Leu Tyr Asp Ala Leu Asp
145                 150                 155                 160
Val Val Leu Tyr Met Asp Pro Met Cys Leu Asp Ala Phe Pro Lys Leu
                165                 170                 175
Val Cys Phe Lys Lys Arg Ile Glu Ala Ile Pro Gln Ile Asp Lys Tyr
            180                 185                 190
Leu Lys Ser Ser Lys Tyr Ile Ala Trp Pro Leu Gln Gly Trp Gln Ala
        195                 200                 205
Thr Phe Gly Gly Gly Asp His Pro Pro Lys Ser Asp Leu Val Pro Arg
    210                 215                 220
Gly Ser Glu Leu Val Ser Cys Thr Thr Val Cys Pro His Ala Gly Lys
225                 230                 235                 240
Ala Lys Ala Glu Lys Val Glu Cys Ala Leu Lys Gly Gly Ile Phe Arg
                245                 250                 255
Gly Thr Leu Pro Ala Ala Asp Cys Pro Gly Ile Asp Thr Thr Val Thr
            260                 265                 270
Phe Asn Ala Asp Gly Thr Ala Gln Lys Val Glu Leu Ala Leu Glu Lys
        275                 280                 285
Lys Ser Ala Pro Ser Pro Leu Thr Tyr Arg Gly Thr Trp Met Val Arg
    290                 295                 300
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Glu Asp Gly Ile Val Glu Leu Ser Leu Val Ser Ser Glu Gln Ser Lys
305                 310                 315                 320
Ala Pro His Glu Lys Glu Leu Tyr Glu Leu Ile Asp Ser Asn Ser Val
                325                 330                 335
Arg Tyr Met Gly Ala Pro Gly Ala Gly Lys Pro Ser Lys Glu Met Ala
            340                 345                 350
Pro Phe Tyr Val Leu Lys Lys Thr Lys Lys
        355                 360
```

Claims

1. A method for assay of anti-Treponema pallidum antibody which comprises assaying anti-Treponema pallidum antibody in a sample by immunoanalysis utilizing the antigen-antibody reaction between G15 antigen, which is a protein available on fusion of glutathione-S-transferase to the N-terminal of a Treponema pallidum membrane antigen having a molecular weight of 15kDa, and/or G17 antigen, which is a protein available on fusion of glutathione-S-transferase to the N-terminal of a Treponema pallidum membrane antigen having a molecular weight of 17kDa, and anti-Treponema pallidum antibody in the sample.

2. The method for assay according to claim 1, wherein the assay is performed by enzyme immunoassay using said G15 antigen and/or said G17 antigen immobilized on a solid phase.

3. The method for assay according to claim 1, wherein the assay is performed by agglutination immunoassay using said G15 antigen and/or said G17 antigen immobilized on a particulate carrier.

Fig. 1

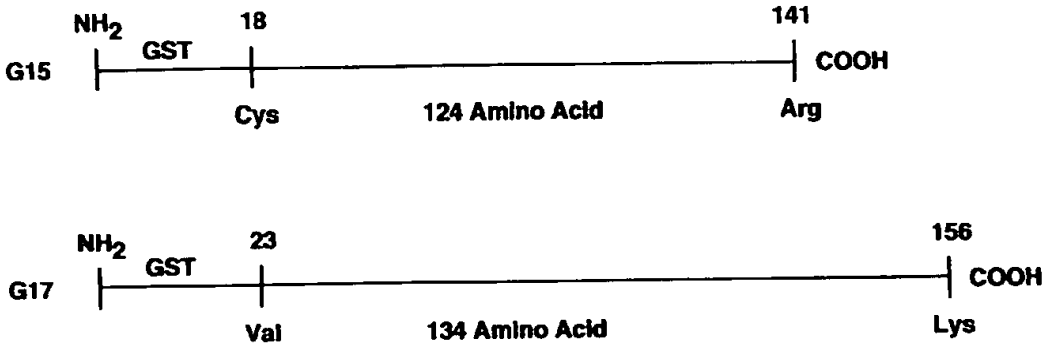

NH2
GST
18
141
G15
COOH
Cys
124 Amino Acid
Arg
NH2
23
156
GST
G17
COOH
Val
134 Amino Acid
Lys

Fig. 2

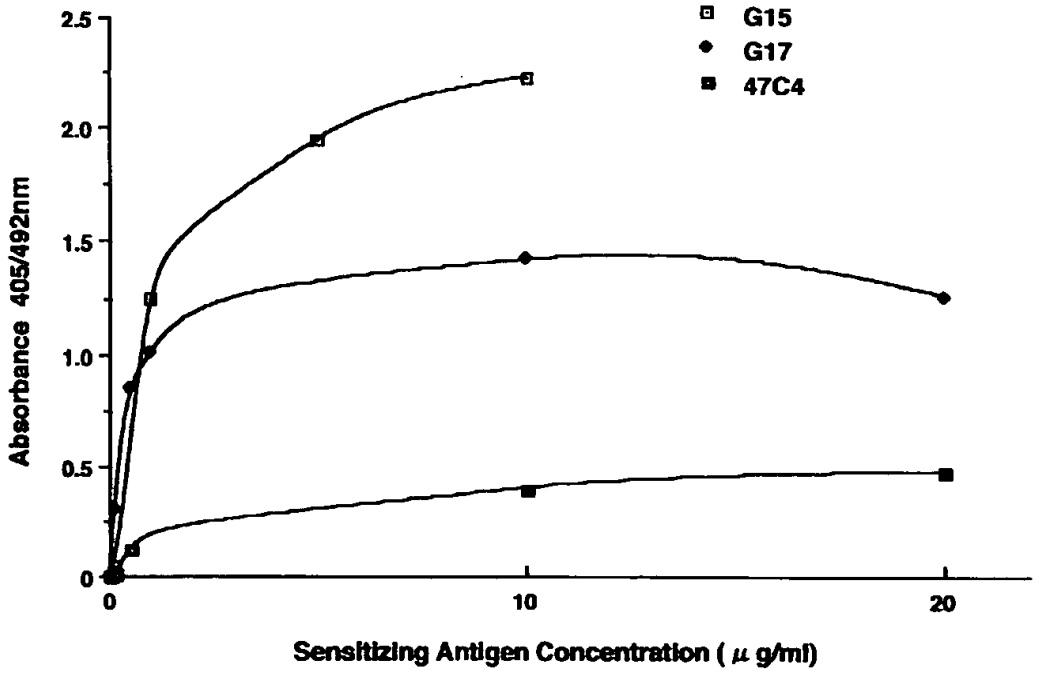

G15
G17
47C4
2.5
2.0
1.5
1.0
0.5
0
Absorbance 405/492nm
0
10
20
Sensitizing Antigen Concentration (μ g/ml)

Fig. 3

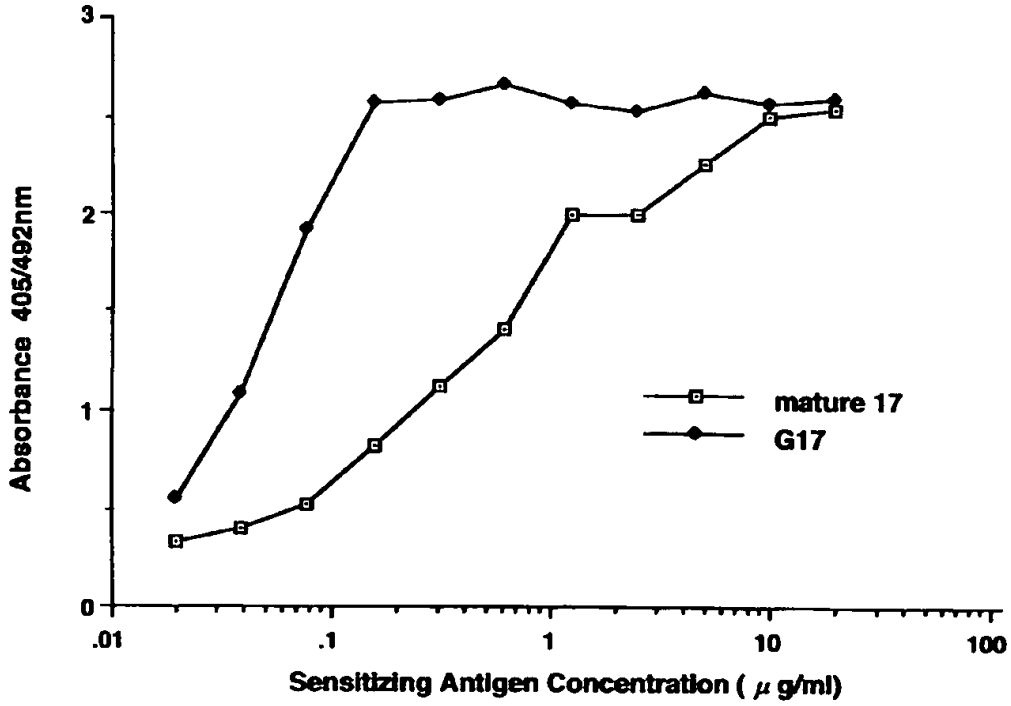

3
2
1
0
Absorbance 405/492nm
.01
.1
1
10
100
Sensitizing Antigen Concentration (μ g/ml)
mature 17
G17

Fig. 4

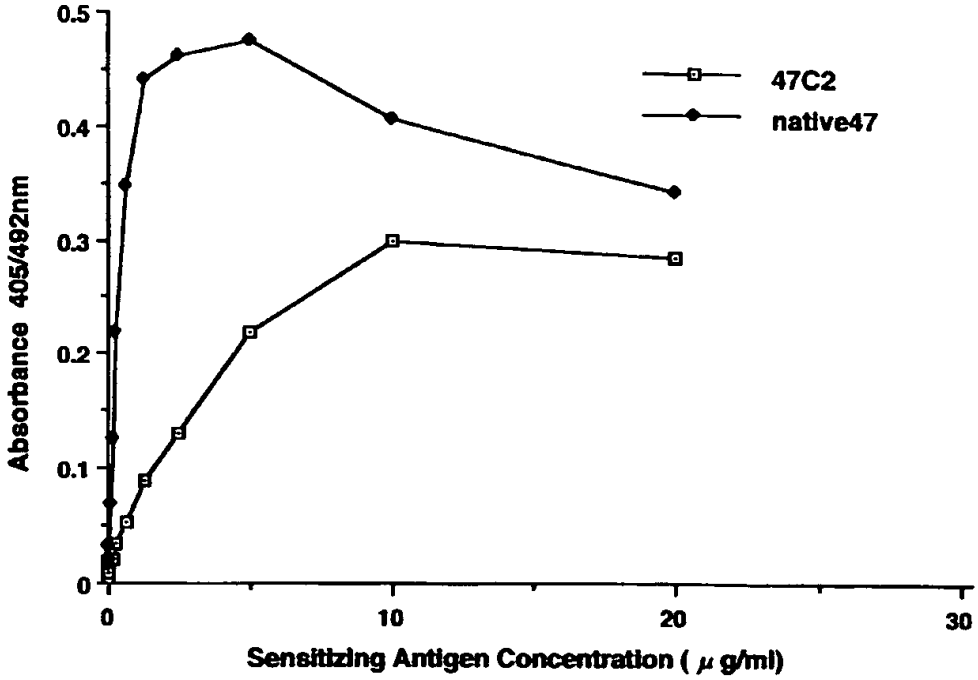

0.5
0.4
0.3
0.2
0.1
0
Absorbance 405/492nm
0
10
20
30
Sensitizing Antigen Concentration (μ g/ml)
47C2
native47

Fig. 5

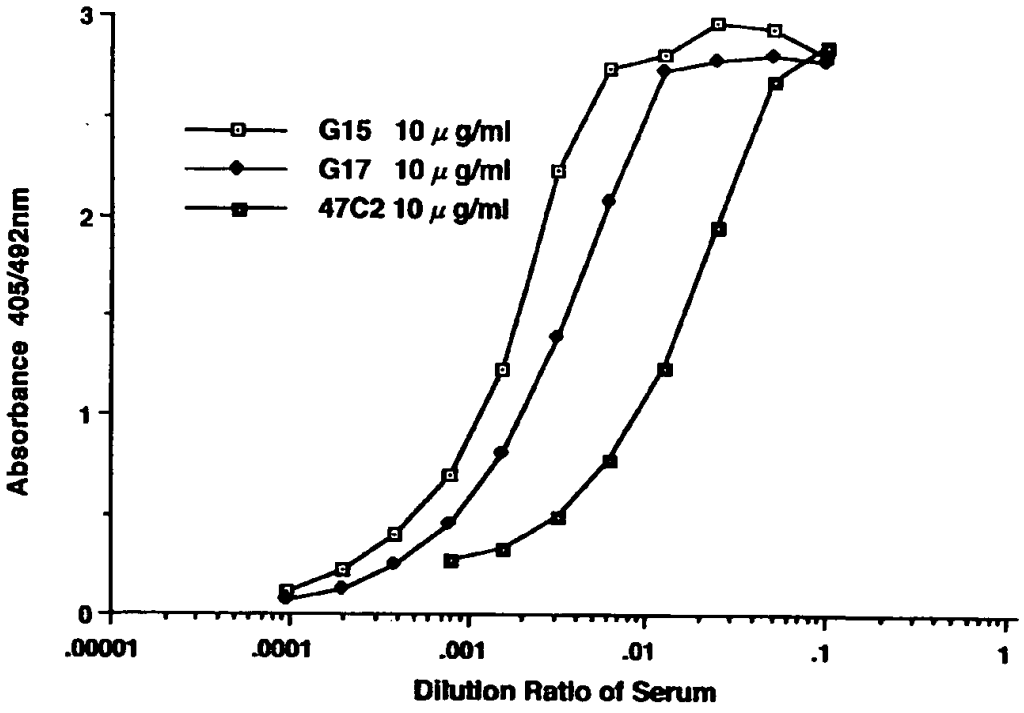

G15 10 μ g/ml
G17 10 μ g/ml
47C2 10 μ g/ml
Absorbance 405/492nm
Dilution Ratio of Serum
0
1
2
3
.00001
.0001
.001
.01
.1
1

Fig. 6

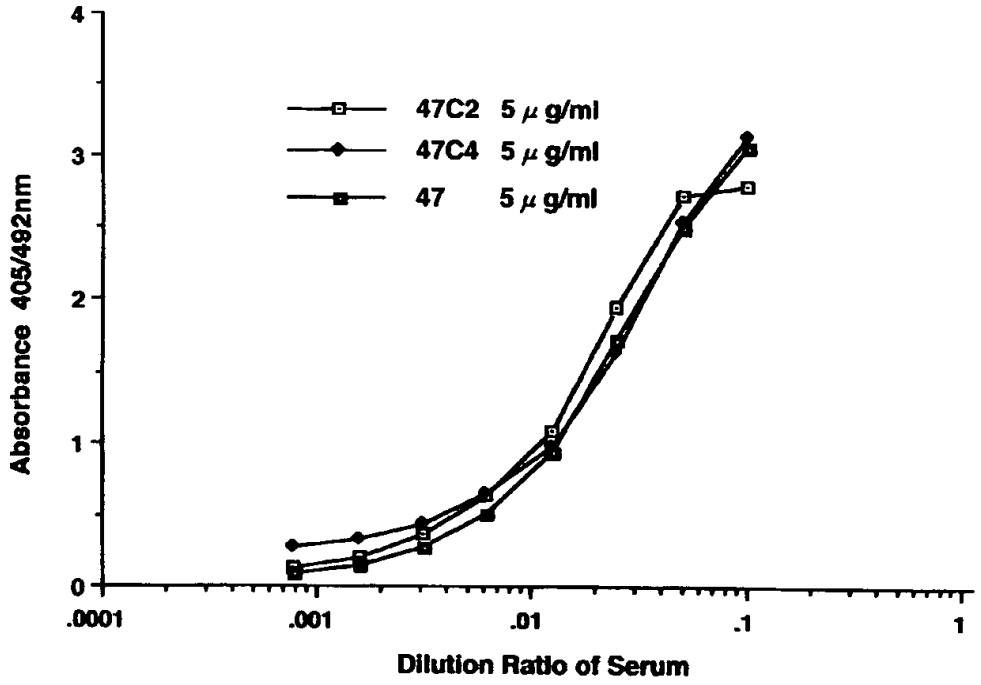
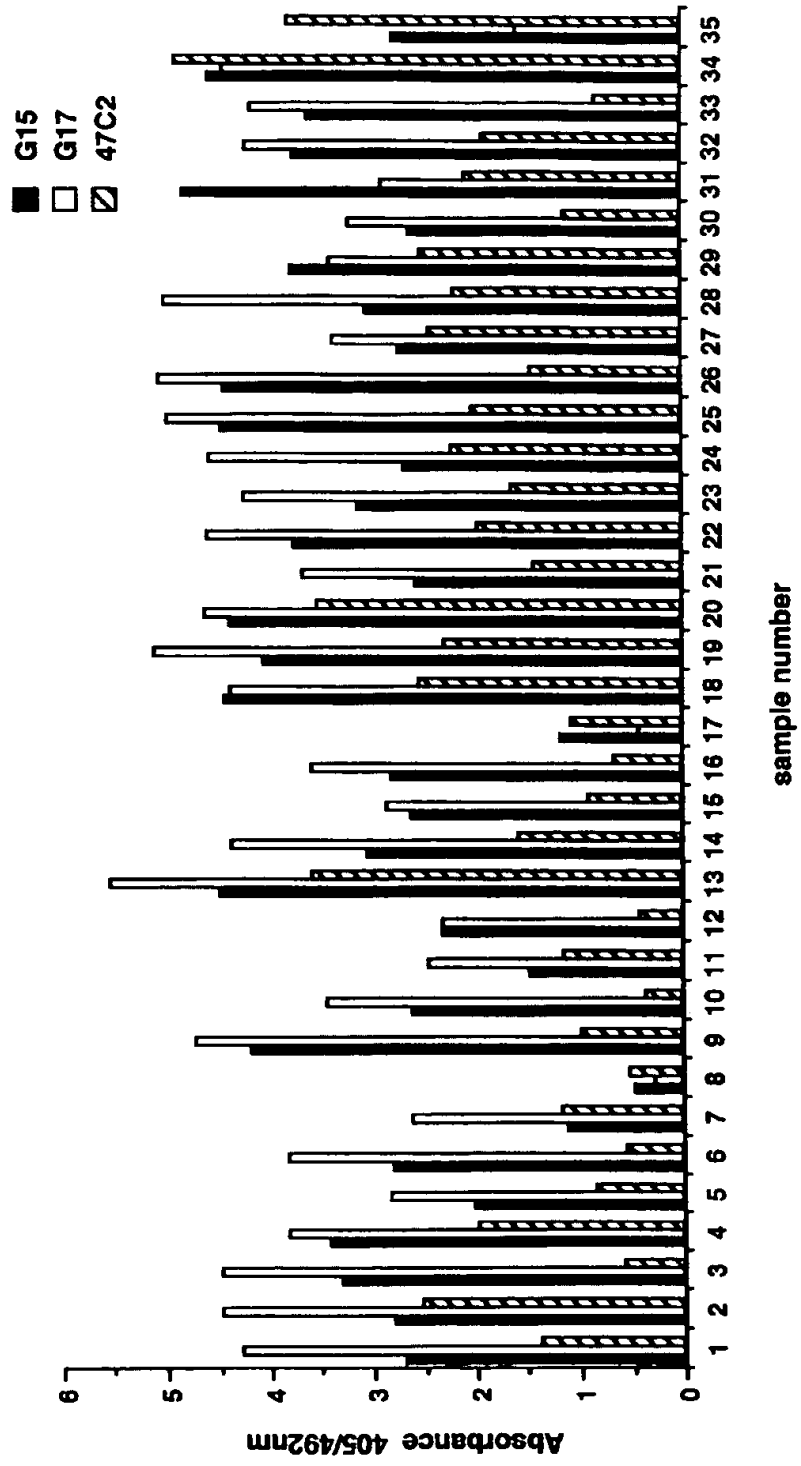

47C2 5 μ g/ml
47C4 5 μ g/ml
47 5 μ g/ml
Absorbance 405/492nm
0
1
2
3
4
.0001
.001
.01
.1
1
Dilution Ratio of Serum

Fig. 7


G15
G17
47C2
Absorbance 405/492nm
0
1
2
3
4
5
6
sample number
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35